

EXHIBIT B, PAGE 1

Page 164:

→ Sacrifice of mice from experiment: adjuvant effect of chemokines (hMCP-4, hMIP-3 α , hTECK Peprotech) on the anti- β gal response (plasmid).

Immunizations: injection of 50 μ l PBS or 50 μ l chemokine diluted in PBS (= 100 ng), and 3 h after footpad injection of 50 μ g (50 μ l) pLacZ or pcDNA3, very low endotoxin (<5 EU).

Same injections at Day 0,7,14,21. Blood sampling for serum at Day 0,14,28.

Day 28: sacrifice of all mice (groups of 6 female BALB/c mice, 6-week old at the beginning of the protocole). Recovery of spleen and popliteal lymph nodes. Single suspension and red blood-cell depletion for spleen.

Cell counts

| | Popliteal lymph node | | spleen | |
|------------------------|-------------------------|---------------------------|-------------------------|---------------------------|
| | Total x 10 ⁶ | /animal x 10 ⁶ | Total x 10 ⁶ | /animal x 10 ⁶ |
| PcDNA3 | 9.66 | 1.61 | 265 | 44.17 |
| PBS + pLacZ | 18.96 | 3.16 | 285 | 47.50 |
| MIP-3 α + pLacZ | 21.6 | 3.6 | 250 | 41.67 |
| MCP-4 + pLacZ | 33 | 5.50 | 325 | 54.17 |
| TECK + pLacZ | 22.8 | 3.8 | 300 | 50.00 |

Culture set up in clone medium + 20 ng IL-2 + 1 μ M BP-1 with /ml: 5 x 10⁶ spleen cells (24 well/group) or 2 x 10⁶ lymph node cells (all cells put in culture).

Day 3: add 1 ml medium + IL-2 + BP-1

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Friday

Experiment irrelevant here

Monday

First experiment irrelevant here

→ ELISA for the adjuvant effect of hMCP-4, hMIP-3 α (not dosed for TECK on that day because not enough coated plates) cf p164 for groups.

Standard protocole for the dosage of IgG, IgG1, IgG2a

- coat 5 μ g/ml, 50 μ l in PBS o/n @ 4°C
- 2 washes PBS
- block 2h @ 37°C
- pooled samples, 1/40 dilution than 1/2 to 1/2 dilutions, triplicates, 1 h @ RT
- anti Igs 1/1000 SAV-PAL 1h
- substrate 30 min
- OD 405-490.

EXHIBIT B, PAGE 2***Page 166:******Raw data tables Day 0 and Day 14***

A: pcDNA3 C: hMIP-3a + pLacZ
B: pLacZ (+PBS) D: hMCP-4 + pLacZ
A1,2,3: dilutions 1/40, 1/80, 1/160 ...

Page 167:***Raw data tables Day 28******Page 168:******Final table***